



EVALUATION OF CHEMICAL CONSTITUENT OF *CITRUS MEDICA LIMONUM* LEAF ESSENTIAL OIL

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ABSTRACT

In the present study *Citrus medica limonum* leaves essential oil was extracted by hydro distillation and the chemical constituents were analyzed by gas chromatography mass spectroscopy (GC-MS) and Fourier transform infrared (FTIR) spectroscopy. Eleven components were identified in the leaf oil by GC-MS, the constituents are dominated by citronellal (29.31 %), limonene (17.59 %), (E)-citral (12.71 %), 1, 6-octadien-3-ol, 3, 7-dimethyl (10.91 %). FTIR identified the functional groups of the oil as C-I, C-H, =C-O-C, C-O, CH₃C-H, C=O, H-C=O, C-H, N-H, CH₃, CH₂.

Keywords: *Citrus medica limonum*, Gas chromatography mass spectroscopy (GC-MS), Fourier transform infrared (FTIR) spectroscopy, Essential oils.

INTRODUCTION

Citrus medica limonum is a small evergreen tree native to Asia, it is a small tree with irregular branches armed with thick spines, stiff and sharp, the leaves are 5 to 7.5 cm long with white flowers that are 2 cm long, the fruits are round and are about 3 cm to 6 cm, greenish yellow, with plenty of acid pulp, and small white oval shaped seeds. The *Citrus medica limonum* tree produces one of the world's most popular citrus fruits and provides a number of other valuable resources. The precise origin of the *Citrus medica limonum* tree is unknown, though Purdue University states that some researchers have linked the tree to Northwestern India. *Citrus medica limonum* trees thrive in warm, coastal climates where the summers are too cold for oranges and grapefruits to ripen. *Citrus medica limonum* is rich in vitamins and minerals, particularly vitamin C, vitamin A, niacin and thiamin. *Citrus medica limonum* leaf is a sedative and antispasmodic used in the cure of insomnia, nervousness, and palpitation¹. *Citrus medica limonum* has anti-catarhal, blood circulation, capillary protector, anti-hypertensive, diuretic, and anti-bacterial and antifungal properties². *Citrus medica limonum* is used around the world in a wide variety of foods and beverages and are often served as garnishes with fish or meat, and with tea, both hot and iced. Additionally, a wide range of sweets are made using *Citrus medica limonum* flavoring, including candies, jams, sherbet, cookies, cakes, puddings, pies, tarts and confectionery². Dehydrated lemon peels are also sold as cattle feed in some parts of the world. *Citrus medica limonum* tree extracts are widely used in cosmetics and beauty products. Petit grain oil made from distilled *Citrus medica limonum* tree twigs, leaves and immature fruits are a common ingredient in floral perfumes and colognes. Many facial cleansing soaps are also made out of lemon juice, and it is reportedly effective in the removal of warts and freckles. Lemon juice is a common ingredient found in cleaning products, as it produces a pleasant, fresh scent, this is due to its high level of acidity, lemon juice is also an effective stain remover. A slice of lemon dipped in salt can be used to clean delicate cooking pots². The essential oil of the whole fruit of *Citrus medica limonum* contains limonene, γ -terpinene, (Z)-citral and (E)-citral and the peel oils of *Citrus medica*

limonum contains limonene and α -terpineol³. *Citrus medica limonum* contains a considerable amount of β -pinene, γ -terpinene, α -terpinolene and trans- α -bergamotene⁴. Marie *et al.*⁵ reported that limonene, γ -terpinene, geraniol, neral were observed for peel oils while leaf oils exhibited the limonene, geraniol, neral composition. In addition *Citrus medica limonum* essential oil contains d-limonene, other terpenes found in the flavedo oil fraction are linalool, geraniol, citronellol, α -terpineol, valencene, mircene, α -pinene, etc.⁶ No report is available regarding *Citrus medica limonum* growing in Zaria. Therefore, the present study represents the first approach for the characterization of the chemical composition of *Citrus medica limonum* leaf essential oils from Zaria. Essential oil of complex composition constitutes limonene, pinene, citral, citronellal, terpineol, camphene, phellandrene, coumarins, flavonoids, vitamin C, carotenoids, mucilage, calcium oxalate. Abundant pectin, sugar, citric acid, malic acid, flavonoids³. Fourier transform infrared (FTIR) spectroscopy has been widely used in food research and has become a powerful analytical tool in the study of edible fats and oils, especially for qualitative identification of specific components in food⁷. FTIR spectroscopy-based methods are fast, sensitive, non destructive, with minimum sample preparation requirement⁸.

MATERIALS AND METHODS

Materials

Fresh leaves were collected from NARICT Zaria in the month of November and identified at the herbarium unit of Biological Science Department A.B.U Zaria to be *Citrus medica limonum* with voucher no 990. The equipments used were Clevenger-type glass apparatus, SHIMADZU FTIR-8400S, GCMS-QP2010 PLUS SHIMADZU, JAPAN.

Methods

Essential Oil Isolation

Fresh leaves were harvested from a healthy, well-grown plant; 300 g of leaves were weighed and chopped into small pieces. The chopped leaves were subjected to hydro-distillation using Clevenger-type glass apparatus⁹. The mixture was boiled vigorously and the distillate was

collected. Finally 0.5 ml of light yellow colored oily liquid with fresh lemon like fragrance was obtained and stored in an air tight container.

G.C Condition for *Citrus medica limonum* Leaves Essential Oils Analysis

Exactly 0.2 ml lemon leaf essential oil was dissolved in 0.8 ml n-Hexane in a 1 mL GC vial and subjected to analysis at suitable conditions.

Instrument- GCMS-QP2010 PLUS SHIMADZU, JAPAN

Purge flow-3.0 mL/min

Column Temperature- 60°C

Injection Temperature-250°C

Mass range- 50-600

Injected volume- 0.2 µl

Table 1: Temperature Program

Rate	Temperature (°C)	Time (min)
-	60.00	1.00
10.00	180.00	3.00
12.00	280.00	2.00

Mass Condition for *Citrus medica limonum* Essential Oils Analysis

Instrument- GCMS-QP2010 PLUS SHIMADZU, JAPAN

Table 2: Temperature Program

Rate	Temperature (°C)	Time (min)
-	3.50	3.50
10.00	200.00	24.00

Identification of the Compounds

The identification of the components was confirmed by comparison of the retention indices with those of authentic compounds and with the NIST 02 library. Percentage composition was computed from GC peak areas on DB-5 ms column without applying correction factors.

FTIR Analysis

Citrus medica limonum leaf oil was subjected to FTIR analysis for the identification of functional groups under the following suitable conditions.

Instrument: SHIMADZU FTIR-8400S Fourier Transform Infrared Spectrophotometer

Wave number range: 500 cm⁻¹ – 4000 cm⁻¹

RESULTS AND DISCUSSION

Table 3 reports the constituents of *Citrus medica limonum* leaves essential oils. Eleven constituents were identified and found to be dominated by citronellal (29.31 %), limonene (17.59 %), (E)-citral (12.71 %), 1,6-octadien-3-ol,3,7-dimethyl (10.91 %), biocyclo [3.1.0] hexane, 4-methylene-1-(1-methyl) (8.80 %), 6-octen-1-ol,3,7-dimethyl (7.95 %), 2,6-octadien-1-ol,3,7-dimethyl-, acetate, (Z) (6.29 %), 1,3-cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl,[S(R*,S*)] (2.81 %), cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)](1.64 %), bezene,1-(1,5-dimethyl-4-hexenyl)-4-methyl (1.10 %) and cyclohexene,1-methyl-4-(5-methyl-1-methyl-1-methylene-4-hexenyl)-(s) (0.88 %).

Table 3: Constituents of leaf essential oil of *Citrus medica limonum*

Peak No.	Name of Components	% Area
1.	Biocyclo[3.1.0] hexane, 4-methylene-1-(1-methyl)	8.80
2.	Limonene	17.59
3.	1,6-octadien-3-ol,3,7-dimethyl	10.91
4.	Citronellal	29.31
5.	6-octen-1-ol,3,7-dimethyl	7.95
6.	(E)-Citral	12.71
7.	2,6-octadien-1-ol,3,7-dimethyl-,acetate,(Z)	6.29
8.	bezene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	1.10
9.	1,3-cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl,[S(R*,S*)]	2.81
10.	Cyclohexene,1-methyl-4-(5-methyl-1-methyl-1-methylene-4-hexenyl)-(s)	0.88
11.	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]	1.64

Linalool, γ -terpinene, (Z)-citral, (E)-citral, citronellal, citronellol, citronellyl acetate, isopulegol, r-cymene, geranial, citronellic acid, α -terpineol, have been reported as major constituents in the leaves and peels oil of others countries^{3,10}. When compared to ours we obtained citronellal, (E)-citral, this confirms that the variations in the cultivar reported is not due to geographic divergence and ecological conditions, it may be due to different chemotype. Gurdip *et al*¹⁰ reported that the major constituents in leaf oil are citronellal, citronellol, limonene, citronellyl acetate, isopulegol, and linalool. On the basis of the above fact it can be concluded that, *Citrus medica limonum* growing widely in zaria, may be utilized as a source for the isolation of natural citronellal and limonene respectively. The high concentration of citronellal and limonene in leaf and peel oil make it potentially useful in the medicines because they exhibit fungi toxicity and repellent properties^{11,12}. The high content of (E)-Citral supports its strong antimicrobial qualities¹³ and pheromonal effects in insects^{14,15}. Gramshaw and Sharpe¹⁶ considered that citral was the key aroma component of citrus odor and flavor and they accepted its content as indicative of the essential oils quality. It indicates that our oil is of good quality due to the presence of citral. The high content of oxygenated compounds in the studied cultivar may explain the powerful and characteristic odor of these plants as is referred to by Lund *et al*.¹⁷

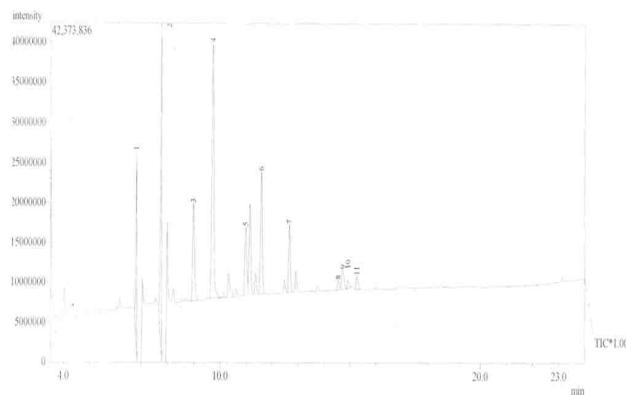


Figure 1: Gas Chromatogram for *Citrus medica limonum* Essential oil

Table 4: MS analysis of *Citrus medica limonum* Essential oil

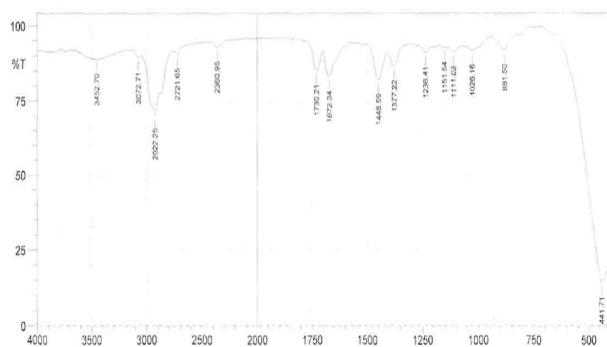
Name of Components	Molecular weight
Biocyclo[3.1.0] hexane, 4-methylene-1-(1-methyl)	136
Limonene	136
1,6-octadien-3-ol,3,7-dimethyl	154
Citronellal	154
6-octen-1-ol,3,7-dimethyl	156
(E)-Citral	152
2,6-octadien-1-ol,3,7-dimethyl-,acetate,(Z)	196
benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	202
1,3-cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl,[S(R*,S*)]	204
Cyclohexene,1-methyl-4-(5-methyl-1-methyl-1-methylene-4-hexenyl)-(s)	204
Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]	204

Table 4 shows the mass spectroscopy of *Citrus medica limonum* essential oil, the components 1,3-cyclohexadiene,5-

(1,5-dimethyl-4-hexenyl)-2-methyl,[S(R*,S*)] and cyclohexene,1-methyl-4-(5-methyl-1-methyl-1-methylene-4-hexenyl)-(s), cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)], were found to have the highest molecular weight of 204 g respectively, benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl had a molecular weight of 196 g, 6-octen-1-ol,3,7-dimethyl had a molecular weight of 156 g, 1,6-octadien-3-ol,3,7-dimethyl and citronellal had a molecular weight of 154 g, (E)-citral had a molecular weight of 152 g, the lowest molecular weight was seen in Biocyclo[3.1.0] hexane, 4-methylene-1-(1-methyl) and Limonene as 136 g. Table 5 shows the identified functional groups of *Citrus medica limonum* leaf oil as C-I, C-H, =C-O-C, C-O, CH₃C-H, C=O,H-C=O, C-H,N-H, CH₃,CH₂. The functional groups were identified by the absorption frequency of the infra red waves in wave number cm⁻¹. The absorption frequencies of each of the functional groups are varied from one another.

Table 5: Functional groups and mode of vibration from FTIR spectra of *Citrus medica limonum* leaf oil

S. NO	Peak position on FTIR spectra (cm ⁻¹)	Class of Compounds	Functional groups	Intensity	Mode of vibration
1.	441.71	Alkyl halides	C-I	Strong	Stretch
2.	881.5	Aromatic and Alkenes	C-H	Strong	Out of plane and bend
3.	1026.16	Ethers	=C-O-C	Medium to Strong	Symmetric Stretch
4.	1111.03	Alcohols	C-O	Medium to Strong	Stretch
5.	1151.54	Alcohols	C-O	Medium to Strong	Stretch
6.	1236.41	Ethers	=C-O-C	Medium to Strong	Asymmetric Stretch
7.	1377.22	Alkanes and Alkyls	CH ₃ C-H	Medium	Bend
8.	1448.59	Alkanes	CH ₃	Medium	Bend
9.	1672.34	Ketones	C=O	Strong	Stretch
10.	1730.21	Aldehydes	C=O	Strong	Stretch
11.	2360.95	Alkanes	CH ₂	Medium	Second over tone
12.	2721.65	Aldehydes	H-C=O	Medium	Stretch
13.	2922.25	Alkanes and Alkyls	C-H	Strong	Stretch
14.	3072.71	Alkenes	C-H	Weak to medium	Stretch
15.	3452.7	Amides	N-H	Weak to medium	Stretch

Figure 2: FTIR chromatogram of *Citrus medica limonum* leaf essential oil

CONCLUSION

The presence of citral and other component and different functional groups indicates that *Citrus medica limonum* leaf essential oil is of good quality

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